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PATENT ABSTRACTS OF JAPAN

(11)Publication number : 10-158265

(43)Date of publication of application : 16.06.1998

(51)Int.Cl. C07D471/16
A61K 49/00
C07K 16/44
G01N 33/50
G01N 33/53

(21)Application number : 08-335107

(71)Applicant : NIPPON ZOKI PHARMACEUT CO LTD

(22)Date of filing : 29.11.1996

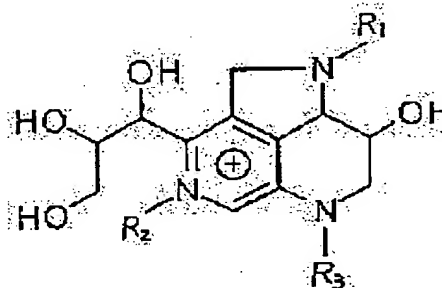
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(54) PYRROLONAPHTHYRIDINIUM DERIVATIVE

(57)Abstract

PROBLEM TO BE SOLVED: To obtain the subject new compound useful for diagnosing diabetes (complications), complications related to dialysis, amyloidosis, aging, diseases, etc., associated with the aging or evaluating pharmacodynamic effects of a medicine effective against the diseases, etc.

SOLUTION: This compound is represented by the formula [R₁ to R₃ are each a [(protected) amino or carboxy-substituted]alkyl] or its salt, e.g. 8⁻



hydroxy-1,2,6,7,8,8a-hexahydro-3-(1,2,3-trihydroxypropyl)-1,4,6tris(3-carboxypropyl)-pyrrolo[2,3,4-de][1,7]naphthyridinium. The compound represented by the formula is obtained by the coexistence of an amine component represented by the formula R₁-NH₂, R₂-NH₂ or R₃-NH₂ with saccharides such as a hexose, an aminosugar or an oligosaccharide.

LEGAL STATUS

[Date of request for examination]

28.11.2003

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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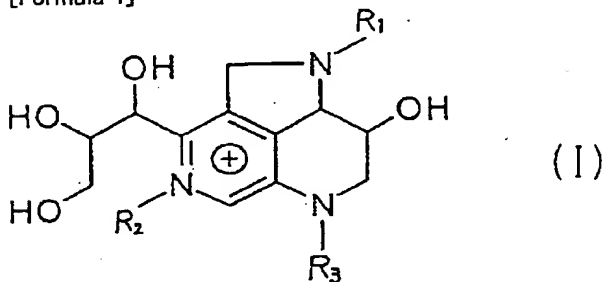
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CLAIMS

[Serial Number] PC-260 [Claim(s)]

[Claim 1] A pyrrolo NAFUCHIRIJINIUMU derivative expressed with a general formula (I), and its salt.

[Formula 1]



R1, R2, and R3 express respectively the same or the alkyl group which may have the amino group which differ and has an amino group and a protective group, and/or a carboxyl group among [type.]

[Claim 2] An antibody created considering a pyrrolo NAFUCHIRIJINIUMU derivative expressed with the above-mentioned general formula (I) as hapten.

[Claim 3] Diagnostics of a disease accompanying diabetes mellitus which made an index a pyrrolo NAFUCHIRIJINIUMU derivative expressed with the above-mentioned general formula (I), diabetic complications, dialysis related complication, amyloidosis, aging, and aging.

[Claim 4] Diagnostics of a disease accompanying diabetes mellitus using an antibody created considering a pyrrolo NAFUCHIRIJINIUMU derivative expressed with the above-mentioned general formula (I) as hapten, diabetic complications, dialysis related complication, amyloidosis, aging, and aging.

[Claim 5] A drug effect appraisal method of diabetic medicine which made an index a pyrrolo NAFUCHIRIJINIUMU derivative expressed with the above-mentioned general formula (I), a diabetic-complications remedy, a dialysis related complication remedy, an amyloidosis remedy, an antiaging drug, and a disease remedy accompanying aging.

[Claim 6] A drug effect appraisal method of diabetic medicine using an antibody created considering a pyrrolo NAFUCHIRIJINIUMU derivative expressed with the above-mentioned general formula (I) as hapten, a diabetic-complications remedy, a dialysis related complication remedy, an amyloidosis remedy, an antiaging drug, and a disease remedy accompanying aging.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technical field to which invention belongs] This invention relates to the drug effect appraisal method of drugs effective in diagnostics, such as a disease accompanying the antibody created considering the new pyrrolo NAFUCHIRIJINIUMU derivative and this derivative as hapten, the diabetes mellitus which used this derivative or its antibody for the list, diabetic complications, dialysis related complication, amyloidosis, aging, and aging, or those diseases.

[0002]

[Description of the Prior Art] The glycosyl hemoglobin (HbA1c) which is the small component of hemoglobin is identified in the living body, it becomes clear that this increases in a diabetic, and the relation between the living thing—meaning of a Maillard reaction especially aging, and diabetes mellitus has come to attract attention ignited by it in 1968. A Maillard reaction is distinguishable to two, an initial stage until the amino group of protein and the aldehyde group of reducing sugar cause and stabilize AMADORI transition after forming a Schiff base, and the later stage which shifts to the Maillard reaction anaphase product (AGE) to which this is characterized by fluorescence, brown change, and molecule bridge formation through a further long-term reaction. The fluorescence known as characteristic change of AGE is [diabetic / healthy person] intentionally high, and the onset and functionality of a diabetic nephropathy, arteriosclerosis, neuropathy, a retinopathy, *****, etc. which are diabetic complication are suggested. Furthermore, recently, the scavenger receptor which recognizes AGE protein exists in monocyte, a macrophage, a mesangial cell, or an endothelial cell, and there is also a report which suggests the relevance of AGE and symptoms, such as inflammation, capillary lock out, and arteriosclerosis, that the AGE recognition through these acceptors causes cytokine emission etc. Moreover, the fluorescence of the protein accumulated into a blood serum increases during dialysis, and the relation of AGE and dialysis related amyloidosis is also pointed out.

[0003]

[Problem(s) to be Solved by the Invention] Although several sorts of AGE candidate material is mentioned until now and those structures are being analyzed, the present condition is that many still unknown points, such as existence of an existence in the living body, a difference in immunochemistry—activity, and relevance with actual symptoms, remain. Unlike the conventional monoamine and a diamine compound, this invention persons found out the new pyrrolo NAFUCHIRIJINIUMU derivative which is the triamine compound which is not reported at all until now, as a result of thinking that important AGE from which structure differs besides the candidate material reported so far will exist and continuing research about AGE further.

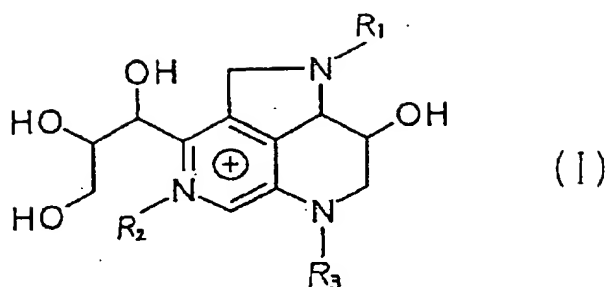
[0004]

[Means for Solving the Problem] The purpose of this invention is to offer a drug effect appraisal method of drugs effective in diagnostics, such as a disease accompanying an antibody created considering a new pyrrolo NAFUCHIRIJINIUMU derivative and this derivative as hapten, diabetes mellitus which used this derivative or its antibody for a list, diabetic complications, dialysis related complication, amyloidosis, aging, and aging, or those diseases.

[0005]

[Embodiment of the Invention] this invention new pyrrolo NAFUCHIRIJINIUMU derivative is a compound expressed with the following general formula (I).

[Formula 2]



R1, R2, and R3 express respectively the same or the alkyl group which may have the amino group which differ and has an amino group and a protective group, and/or a carboxyl group among [type.]

R1 of the above-mentioned general formula (I), and R2 And R3 As an alkyl group which can be set, the carbon number 1 of the shape of straight chains, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, t-butyl, pentyl, isopentyl, neopentyl one, t-pentyl, hexyl, and dimethyl butyl, or branching thru/or the alkyl group of 6 are mentioned preferably.

[0006] Said alkyl group may have the amino group and/or carboxyl group which have an amino group and a protective group. As a protective group of the amino group The protective group usually used in fields, such as peptide synthetic chemistry, can be used. For example, acetyl, benzyloxycarbonyl, p-methoxybenzyloxy carbonyl, p-chloro benzyloxycarbonyl, p-nitro benzyloxycarbonyl, p-phenylazo benzyloxycarbonyl, p-methoxy phenylazo benzyloxycarbonyl, Radicals, such as t-butoxycarbonyl (Boc), p-tosyl (Tos), the third friend ROKISHI carbonyl, p-biphenyl isopropoxy carbonyl, diisopropyl MECHIROKISHIKABONIRU, and formyl, are mentioned.

[0007] In this invention material expressed with a general formula (I), it is R1 and R2. And R3 The pyrrolo NAFUCHIRIJINIUMU derivative which is the alkyl group which has an amino group and/or a carboxyl group is useful, especially when it can be made to combine with carrier protein etc. easily as hapten and an antibody is created. As support combined with hapten in order to create an antibody, support usually used, such as polymer, such as protein, such as serum albumin and limpet blood pigment protein, and the poly lysine, can be used.

[0008] this invention pyrrolo NAFUCHIRIJINIUMU derivative includes the salt expressed with the aforementioned general formula (I). For example, a hydrochloric acid, a sulfuric acid, a nitric acid, a hydrobromic acid, a phosphoric acid, perchloric acid, thiocyanic acid, A boric acid, a formic acid, an acetic acid, a halo acetic acid, a propionic acid, a glycolic acid, a citric acid, A tartaric acid, a succinic acid, a gluconic acid, a lactic acid, a malonic acid, a fumaric acid, an anthranilic acid, A benzoic acid, a cinnamic acid, p-toluenesulfonic acid, a naphthalene sulfonic acid, A salt with a metal with alkaline earth metal, such as alkali metal, such as an addition salt with organic bases, such as an addition salt with an acid with a sulfanilic acid etc., ammonia, and an organic amine, or sodium, and a potassium, calcium, magnesium, and barium, or aluminum, zinc, etc. is mentioned. These salts can be manufactured from this invention pyrrolo NAFUCHIRIJINIUMU derivative of isolation by the well-known method, or can be changed mutually.

[0009] Moreover, when stereoisomers, such as a cis—transformer object, an optical isomer, and a conformer, exist in this invention compound, or when it exists in the state of a hydrate or a complex compound, this invention also includes which the stereoisomer, a hydrate, and a complex compound.

[0010] Next, an example of the manufacture method of this invention compound is described. R1-NH2 and R2-NH2 Or R3-NH2 this invention compound can be obtained by making the compound (amine component: R1, R2, and R3 expressing the same radical as the above respectively) expressed coexist with saccharides, such as oligosaccharides, such as aminosugar, such as hexose, such as a glucose, fructose, a galactose, a mannose, and deoxyglucose, a glucosamine, and galactosamine, and saccharose. Moreover, after carrying out a mixed reaction, using protein and peptides as an amine component, acidolysis processing can be performed and this invention compound can also be obtained.

[0011] About reaction conditions, such as reaction, temperature, reaction time, and pH, there are no special setups and they can be set up suitably. Although what is necessary is just to leave easy one in the room temperature on actuation, a reaction can be promoted by heating etc. The usual means, such as distillation, a chromatography, and recrystallization, can refine the obtained this invention compound.

[0012]

[Example] This invention is not limited by this although the example of this invention pyrrolo NAFUCHIRIJINIUMU derivative is shown below.

It dissolved in 1100ml (pH7.3) of 250mM phosphate buffer solutions, and example 1. glucose 79.2g and 45.3g (gamma-aminobutyric acid) of gamma-aminobutyric acid were put for 45 days at 37 degrees C. The reaction solution was added to sulfonic acid type cation exchange resin / DIAION PK-216 (Mitsubishi Kasei), it was [bottom 40 degree-C of reduced pressure] under water bath, and concentration hardening by drying of the solution eluted with 2-N aqueous ammonia was carried out after rinsing. AMBERLITE XAD-2 which equilibrated with ion exchange water after dissolving a hardening-by-drying object in little ion exchange water It added in the column (ORGANO CORP.)

and passage fractions were collected. DEVELOSIL ODS LOP-45S after condensing this fraction It added in the column (Nomura chemistry) and was eluted with the methanol-trifluoroacetic acid mixed solution. Furthermore, separation purification of this was carried out with reversed phase high pressure liquid chromatography / STR ODS-II column (Shimazu techno research), and the compound 1 (65mg), the compound 2 (182.9mg), and compound 3 (35.5mg) which are a stereoisomer were isolated, respectively.

[0013] Example 2. glucose 25.2g and alpha-acetyl lysine 34g were dissolved in 700ml of 250mM phosphate buffer solutions, the same actuation as an example 1 was performed, and the compound 4 (2mg) and compound 5 (3mg) which are a stereoisomer were isolated, respectively.

[0014] The physical-properties value of the obtained this invention compound is shown below. In addition, the quality of a label ghost was manufactured using the glucose to which the label of the 1st place or the total carbon was carried out by ^{13}C by the same method as the above-mentioned example, and it used for structural analysis. Fluorescence spectrum Both ultraviolet-region absorption (UV) spectrums were measured in the methanol by DU-650 (Beckman) with 650-40 Fluorescence Spectrophotometer (Hitachi). Spatter DOION mass analysis PEKUTORU (SIMS) The glycerol was used for the matrix by M-80B (Hitachi), and it measured. The nuclear-magnetic-resonance (NMR) spectrum was measured by ARX-500 (Bruker) among heavy water, and, in the proton, ^{13}C set the resonance frequency of 125.77MHz to 0.00 ppm for the resonance frequency of 500.13MHz. Attribution of ^1H -NMR spectrum and ^{13}C -NMR spectrum ^1H - ^1H Two-dimensional NMR, such as COSY and HMQC, determined.

[0015] - compound 18-hydroxy-1, 2, 6, 7 and 8, and 8a-hexahydro-3-(1, 2, 3-trihydroxy propyl)- 1, 4, and 6-tris (3-carboxy propyl)-pyrrolo [2, 3, 4-de] [1, 7] NAFUCHIRIJINIUMU fluorescence-spectrum:EXmax = 370 nm, EMmax = 450 nmUV spectrum:lambda max = 237, 276, 360 nmSIMS:m/z 5271 H-NMR(D₂O)-deltappm: 1.79 (2H, m, H-2''), 2.04 (2H, m, H-2'), 2.13 (2H, m, H-2'') 2.33 (2H, t, J = 7Hz, H-3''), 2.44 (4H, t, J = 7Hz, H-3', 3'') 3.35 (2H, m, H-1''), 3.47 (1H, m, H-1') 3.50 (1H, dd, J = 14 or 2Hz, H-7), 3.59 (1H, m, H-1') 3.63-3.69 (4H, m, H-7, 10, 11, 11), 4.33 (1H, m, H-1'') 4.68 (1H, brs, H-8), 4.68 (1H, m, H-1'') 4.83 (1H, d, J = 14Hz, H-2), 4.98 (1H, d, J = 1Hz, H-8a) 5.20 (1H, d, J = 9Hz, H-9), 5.36 (1H, dd, J = 14 or 1Hz, H-2), 7.92 (1H, s, H-5) ^{13}C -NMR(D₂O)-deltappm : 22.2 (t, C-2', 2''), 27.1 (t, C-2''), 31.5 (t, C-3''), 32.2 (t, C-3'), 33.3 (t, C-3''), 49.4 (t, C-1''), 53.5 (t, C-7), 54.5 (t, C-1'), 58.6 (d, C-8), 59.8 (t, C-2, 1'), 63.2 (t, C-11), 67.3 (d, C-8a), 68.6 (d, C-9), 74.6 (d, C-10), 126.8 (d, C-5), 134.6 (s and C-2a), 135.3 (s and C-8b), 139.7 (s, C-3), 140.8 (s and C-5a), 178.8 (s, C-4''), 180.2 (s and C-4', 4'') [0016] - compound 28-hydroxy-1,

2, 6, 7 and 8, and 8a-hexahydro-3-(1, 2, 3-trihydroxy propyl)- 1, 4, and 6-tris (3-carboxy propyl)-pyrrolo [2, 3, 4-de] [1, 7] NAFUCHIRIJINIUMU fluorescence-spectrum:EXmax = 370 nm, EMmax = 450nmUV spectrum:lambda max = 240, 277, 360 nmSIMS:m/z 5271 H-NMR(D₂O)-deltappm: 1.82 (2H, m, H-2''), 2.06 (2H, m, H-2') 2.12 (2H, m, H-2''), 2.35 (2H, t, J = 7Hz, H-3'') 2.46 (4H, t, J = 7Hz, H-3', 3''), 3.33 (1H, ddd, J = 14, 7 or 7Hz, H-1''), 3.42 (1H, ddd, J = 14, 7 or 7Hz, H-1''), 3.48 (1H, m, H-1') 3.49 (1H, dd, J = 14 or 2Hz, H-7), 3.61 (1H, m, H-1') 3.68 (1H, dd, J = 14 or 1Hz, H-7), 3.69 (1H, ddd, J = 9, 3 or 3Hz, H-10) 3.73 (1H, dd, J = 3 or 3Hz, H-11), 3.76 (1H, dd, J = 3 or 3Hz, H-11) 4.39 (1H, m, H-1''), 4.68 (1H, d, J = 2Hz, H-8) 4.79 (1H, m, H-1''), 4.89 (1H, d, J = 14Hz, H-2) 5.01 (1H, d, J = 2Hz, H-8a), 5.06 (1H, d, J = 14Hz, H-2) 5.21 (1H, d, J = 9Hz, H-9), 7.95 (1H, s, H-5) ^{13}C -NMR(D₂O)-deltappm: 21.8 (t, C-2''), 22.2 (t, C-2'), 26.8 (t, C-2''), 31.5 (t, C-3''), 31.8 (t, C-3'), 32.0 (t, C-3''), 49.8 (t, C-1''), 53.9 (t, C-7), 54.3 (t, C-1'), 58.8 (t, C-1'), 60.4 (d, C-8), 61.1 (t, C-2), 63.7 (t, C-11), 67.7 (d, C-8a), 69.0 (d, C-9), 74.8 (d, C-10), 127.3 (d, C-5), 134.5 (s and C-2a), 135.1 (s and C-8b), 141.1 (s, C-3, 5a), 178.0 (s and C-4', 4''), 178.8 (s and C-4'') [0017] - compound

38-hydroxy-1, 2, 6, 7 and 8, and 8a-hexahydro-3-(1, 2, 3-trihydroxy propyl)- 1, 4, and 6-tris (3-carboxy propyl)-pyrrolo [2, 3, 4-de] [1, 7] NAFUCHIRIJINIUMU fluorescence-spectrum:EXmax = 373 nm, EMmax = 452 nmUV spectrum:lambda max = 239, 278, 360 nmSIMS:m/z 5271 H-NMR(D₂O)-deltappm: 1.84 (2H, m, H-2'') 2.03 (2H, m, H-2'), 2.12 (2H, m, H-2'') 2.35 (2H, t, J = 7Hz, H-3''), 2.46 (4H, m, H-3', 3'') 3.31 (1H, ddd, J = 14, 7 or 7Hz, H-1''), 3.39 (1H, ddd, J = 14, 7 or 7Hz, H-1''), 3.46 (1H, dd, J = 14 or 1Hz, H-7) 3.50 (1H, m, H-1'), 3.78 (1H, dd, J = 14 or 3Hz, H-7) 3.70-3.79 (4H, m, H-10, 11 and 11, 1'), 4.32 (2H, m, H-8, 1'') 4.78 (1H, m, H-1''), 4.79 (1H, d, J = 10Hz, H-8a) 4.83 (1H, d, J = 14Hz, H-2), 5.08 (1H, d, J = 14Hz, H-2) 5.22 (1H, d, J = 9Hz, H-9), 7.93 (1H, s, H-5) ^{13}C -NMR(D₂O)-deltappm: 21.8 (t, C-2''), 22.1 (t, C-2'), 26.6 (t, C-2''), 31.3 (t, C-3''), 31.6 (t, C-3'), 31.8 (t, C-3''), 49.6 (t, C-1''), 54.0 (t, C-7), 55.7 (t, C-1'), 60.2 (t, C-2), 60.4 (t, C-1''), 63.5 (t, C-11), 63.9 (d, C-8), 69.0 (d, C-9), 70.4 (d, C-8a), 74.7 (d, C-10), 127.7 (d, C-5), 134.1 (s and C-2a), 135.2 (s and C-8b), 141.4 (s and C- 5a, 3), 177.7 (s, C-4''), 177.7 (s and C-4'), 178.8 (s and C-4'') [0018] - compound 48-hydroxy-1, 2, 6, 7 and 8, and 8a-hexahydro-3-(1, 2,

3-trihydroxy propyl)- 1, 4, and 6-tris [6-(N-acetyl-L-NORUROISHIRU)]-pyrrolo [2, 3, 4-de] [1, 7] NAFUCHIRIJINIUMU fluorescence-spectrum:EXmax = 374 nm, EMmax = 452 nmUV spectrum:lambda max = 237, 277, and 360 nmSIMS:m/z 7821 H-NMR(D₂O)-deltappm: 1.40 (6H, m, H-3', 3'', and 3' — ') — 1.68 (2H, m, H-2'') 1.72 (4H, m, H-2', 2''), 1.84 (6H, m, H-4', 4'', and 4' — ') and 1.98 (3H, s, AcO' — Me) — 1.99 (3H, s, AcO' — Me) 2.00 (3H, s, AcO' — Me), 3.41 (1H, m, H-1') 3.53 (2H, m, H-1''), 3.61 (1H, dd, J = 14 or 1Hz, H-7) 3.62 (1H, m, H-1'), 3.74 (1H, dd, J = 14 or 1Hz, H-7) 3.79 (11 3H, m, H-10, 11), 4.23 (3H, m, H-5', 5'', and 5' — ') and 4.42 (1H, m, H-1'') — 4.70 (1H, s, H-8) 4.70 (1H, m, H-1''), 4.89 (1H, d, J = 14Hz, H-2), 5.07 (1H, d, J = 1Hz, H-8a), 5.22 (1H, d, J = 9Hz, H-9), 5.41 (1H, dd, J = 14 or 1Hz, H-2), 7.95 (1H, s, H-5) ^{13}C -NMR(D₂O)-deltappm: 22.6 (q, AcO' [— Me] — Me, AcO' — Me, AcO''), 22.8(t,C-2'), 23.1(t,C-2'',3',3''), 25.4(t,C-3''), 26.0(t,C-2''), 30.8(t,C-4'), 30.9(t,C-4''), 31.3(t,C-4'') 49.9(t,C-1'), 53.4(d,C-5',5''), 53.6(d,C-5''), 54.6(t,C-7,1''), 58.5(d,C-8), 60.5(t,C-1''), 60.8(t,C-2), 63.1(t,C-11), 67.3(d,C-8a), 68.6(d,C-9),74.4 (d, C-10), 126.6 (d, C-5), 134.0 (s and C-2a), 134.3 (s and C-8b), 140.1 (s, C-3), 140.7 (s and C-5a), 175.1 (s and C-6'), 175.1 (s, C-6''), 175.2 (s and C-6''), 176.6 (s and AcO'-CO), 176.7 (s, AcO''-CO), 176.8 (s, AcO''-CO) [0019] - compound 58-hydroxy-1, 2, 6, 7 and 8, and 8a-hexahydro-3-(1, 2, 3-trihydroxy

propyl)- 1, 4, and 6-tris [6-(N-acetyl-L-NORUROISHIRU)]-pyrrolo [2, 3, 4-de] [1, 7] NAFUCHIRIJINIUMU
 fluorescence-spectrum:EXmax = 376 nm, EMmax = 452 nmUV spectrum:lamdamax = 239, 278, and 360 nmSIMS:m/z
 7821 H-NMR(D2O)-deltappm: 1.39-1.48 (6H, m, H-3', 3'', and 3''' — ') — 1.66 (2H, m, H-2'') 1.73 (4H, m, H-2', 2''),
 1.89 (6H, m, H-4', 4'', and 4''' — ') and 1.98 (3H, s, AcO'' — Me) — 1.98 (3H, s, AcO' — Me) 2.00 (3H, s, AcO'' — Me),
 3.39 (1H, m, H-1'') 3.44 (1H, m, H-1'''), 3.50 (1H, m, H-1') 3.57 (1H, dd, J = 14 or 2Hz, H-7), 3.65 (1H, m, H-1') 3.73
 (1H, dd, J = 14 or 1Hz, H-7), 3.81 (11 3H, m, H-10, 11) and 4.27-4.35 (four — H — m — H — one — '' — five — ' —
 five — '' — five — '' — ') — 4.75 (1H, brs, H-8) 4.78 (1H, m, H-1''), 4.94 (1H, d, J= 14Hz, H-2), 5.08 (1H, brs, H-8a),
 5.09 (1H, dd, J = 14 or 1Hz, H-2), 5.20 (1H, d, J= 9Hz, H-9), 7.95 (1H, s, H-5) [0020]

[Effect of the Invention] Some candidate material is reported as AGE until now, and research is advanced also for
 current. Although this invention person and its associate had also found out a pyridinium derivative and a
 NAFUCHIRIJINIUMU derivative which are indicated by JP,6-73057,A and JP,8-48686,A, these made the
 condensation 2 ring system of diamine the basic mother nucleus. Unlike the conventional compound, this invention
 pyrrolo NAFUCHIRIJINIUMU derivative should make the condensation 3 ring system of triamine a basic mother
 nucleus, and should be observed very much as completely new AGE candidate material from the new structure of a
 flume lie with three parts in which bridge formation with protein in the living body is possible.

[0021] Therefore, like the AGE compound reported conventionally, this invention compound is made into an index, a
 diagnosis of aging, the disease accompanying it, etc. is possible in diabetic complication lists, such as diabetes
 mellitus and diabetic nephropathy, diabetic arteriosclerosis, a diabetic neuropathy, diabetic cataract, diabetic
 retinopathy, and a diabetic microangiopathy, and pharmacometrics etc. can be further performed by making this
 invention compound into an index in in vitro one and an in vivo trial system. Moreover, the antibody created as
 hapten can use this invention compound immunochemistry-wise and in immunohistochemistry in the
 above-mentioned diagnosis or pharmacometrics, and usefulness is very high. As mentioned above, known AGE is new
 material which is the condensation 3 ring system frame of clearly different triamine, the existence [in the living body
] and bioactive different from old AGE candidate material are suggested, and this invention pyrrolo
 NAFUCHIRIJINIUMU derivative can also expect different usefulness.

[Translation done.]

(19) 日本国特許庁(JP)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平 10-158265

(43) 公開日 平成10年(1998)6月16日

(51) Int Cl^o

識別記号

F I

C 0 7 D 471/16

C 0 7 D 471/16

A 6 1 K 49/00

A 6 1 K 49/00

A

C 0 7 K 16/44

C 0 7 K 16/44

G 0 1 N 33/50

G 0 1 N 33/50

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審査請求 未請求 請求項の数6

F D

(全5頁)

(21) 出願番号

特願平 8-335107

(22) 出願日

平成8年(1996)11月29日

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(54) 【発明の名称】 ピロロナフチリジニウム誘導体

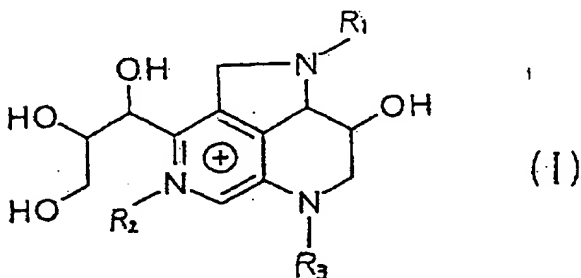
(57) 【要約】

(修正有)

【課題】糖尿病、糖尿病合併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患等の診断などに有効な新規なピロロナフチリジニウム誘導体及び該誘導体をハプテンとして作成された抗体を提供する。

【解決手段】本発明ピロロナフチリジニウム誘導体は次の一般式で表される新規化合物である。

【化1】



〔式中、R₁、R₂及びR₃は各々同一若しくは異なつてアミノ基、保護基を有するアミノ基及び／又はカルボキシル基を有してもよいアルキル基を表す。〕

【効果】本発明化合物を指標として、糖尿病、糖尿病合

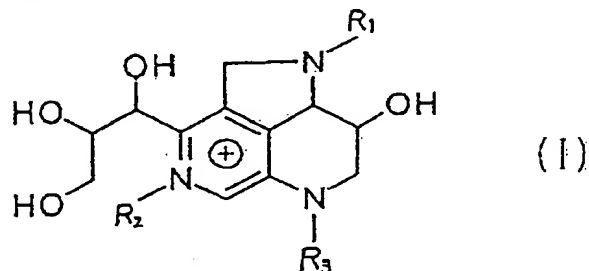
併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患等の診断が可能であり、またそれら疾患等に有効な薬剤の薬効評価法に利用することもできる。

【整理番号】 PC-260

【特許請求の範囲】

【請求項1】 一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体及びその塩。

【化1】



【式中、R₁、R₂及びR₃は各々同一若しくは異なつてアミノ基、保護基を有するアミノ基及び/又はカルボキシル基を有してもよいアルキル基を表す。】

【請求項2】 上記一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体をハプテンとして作成された抗体。

【請求項3】 上記一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体を指標とした糖尿病、糖尿病合併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患の診断法。

【請求項4】 上記一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体をハプテンとして作成された抗体を用いた糖尿病、糖尿病合併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患の診断法。

【請求項5】 上記一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体を指標とした糖尿病治療薬、糖尿病合併症治療薬、透析関連合併症治療薬、アミロイドーシス治療薬、老化防止薬、老化に伴う疾患治療薬の薬効評価法。

【請求項6】 上記一般式(Ⅰ)で表されるピロロナフチリジニウム誘導体をハプテンとして作成された抗体を用いた糖尿病治療薬、糖尿病合併症治療薬、透析関連合併症治療薬、アミロイドーシス治療薬、老化防止薬、老化に伴う疾患治療薬の薬効評価法。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】 本発明は、新規なピロロナフチリジニウム誘導体及び該誘導体をハプテンとして作成された抗体、並びに該誘導体又はその抗体を用いた糖尿病、糖尿病合併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患等の診断法或いはそれらの疾患等に有効な薬剤の薬効評価法に関する。

【0002】

【従来の技術】 1968年、ヘモグロビンの小成分であるグリコシルヘモグロビン(HbA1c)が生体内で同

定され、これが糖尿病患者において増加することが判明し、それを契機にメイラード反応の生物学的意義、特に老化と糖尿病との関係が注目されるようになってきた。メイラード反応は蛋白のアミノ基と還元糖のアルデヒド基とが Schiff 塩基を形成後、アマドリ転移を起こして安定化するまでの初期段階と、これがさらに長期の反応を経て、蛍光、褐色変化、分子架橋を特徴とするメイラード反応後期生成物(AGE)に移行する後期段階の2つに区別できる。AGEの特徴的变化として知られている蛍光性は糖尿病患者では健常者に比べて有意に高く、また糖尿病の合併症である糖尿病性の腎症、動脈硬化症、神経障害、網膜症、白内障等の発症と相関性が示唆されている。更に最近では、単球、マクロファージ、メサングウム細胞や内皮細胞にAGE蛋白質を認識するスカベンジャーレセプターが存在し、これらの受容体を介したAGE認識がサイトカイン放出等を引き起こすという、AGEと炎症、毛細血管閉塞、動脈硬化等の病態との関連性を示唆する報告もある。また、透析中に血清中に蓄積する蛋白の蛍光が増加し、AGEと透析関連アミロイドーシスとの関連も指摘されている。

【0003】

【発明が解決しようとする課題】 これまで数種のAGE候補物質が挙げられており、それらの構造は解析されつつあるが、生体内における存在の有無、免疫化学的活性の差異、実際の病態との関連性など未だ不明な点が多く残っているのが現状である。本発明者らは、これまで報告されてきた候補物質以外にも構造の異なる重要なAGEが存在するのではないかと考え、更にAGEに関して研究を続けた結果、従来のモノアミン、ジアミン化合物とは異なり、これまでに全く報告されていないトリアミン化合物である新規なピロロナフチリジニウム誘導体を見出した。

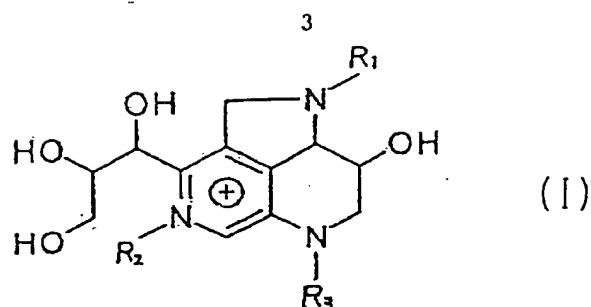
【0004】

【課題を解決するための手段】 本発明の目的は、新規なピロロナフチリジニウム誘導体及び該誘導体をハプテンとして作成された抗体、並びに該誘導体又はその抗体を用いた糖尿病、糖尿病合併症、透析関連合併症、アミロイドーシス、老化、老化に伴う疾患等の診断法或いはそれらの疾患等に有効な薬剤の薬効評価法を提供することにある。

【0005】

【発明の実施の形態】 本発明新規ピロロナフチリジニウム誘導体は次の一般式(Ⅰ)で表される化合物である。

【化2】



〔式中、 R_1 、 R_2 、及び R_3 は各々同一若しくは異なつてアミノ基、保護基を有するアミノ基及び／又はカルボキシル基を有してもよいアルキル基を表す。〕

上記一般式(I)の R_1 、 R_2 、及び R_3 におけるアルキル基としては、好ましくはメチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec-ブチル、t-ブチル、ペンチル、イソペンチル、ネオペンチル、t-ペンチル、ヘキシル、ジメチルブチル等の直鎖又は分枝状の炭素数1乃至6のアルキル基が挙げられる。

【0006】前記アルキル基は、アミノ基、保護基を有するアミノ基及び／又はカルボキシル基を有していてもよく、アミノ基の保護基としては、ペプチド合成化学等の分野で通常使用されている保護基が利用でき、例えば、アセチル、ベンジルオキシカルボニル、p-メトキシベンジルオキシカルボニル、p-クロロベンジルオキシカルボニル、p-ニトロベンジルオキシカルボニル、p-フェニルアゾベンジルオキシカルボニル、p-メトキシフェニルアゾベンジルオキシカルボニル、t-ブトキシカルボニル(Boc)、p-トルエンシルホン(Tos)、第三アミロキシカルボニル、p-ビフェニルイソプロピルオキシカルボニル、ジイソプロピルメチロキシカルボニル、ホルミル等の基が挙げられる。

【0007】一般式(I)で表される本発明物質のなかで、 R_1 、 R_2 、及び R_3 がアミノ基及び／又はカルボキシル基を有するアルキル基であるピロロナフチリジニウム誘導体は、ハプテンとして担体蛋白質等と容易に結合させることができ、抗体を作成するときには特に有用である。抗体を作成するためにハプテンと結合させる担体としては、血清アルブミン、カサガイ血液色素蛋白質等の蛋白質やポリリジン等のポリマー類など通常使用されている担体類を用いることができる。

【0008】本発明ピロロナフチリジニウム誘導体は、前記の一般式(I)で表される塩を包含し、例えば、塩酸、硫酸、硝酸、臭化水素酸、リン酸、過塩素酸、チオシアン酸、ホウ酸、ギ酸、酢酸、ハロ酢酸、プロピオン酸、グリコール酸、クエン酸、酒石酸、コハク酸、グルコン酸、乳酸、マロン酸、フマル酸、アントラニル酸、安息香酸、ケイ皮酸、p-トルエンシルホン酸、ナフタレンシルホン酸、スルファニル酸等との酸との付加塩、

アンモニア、有機アミン等の有機塩基との付加塩、或いはナトリウム、カリウム等のアルカリ金属、カルシウム、マグネシウム、バリウム等のアルカリ土類金属又はアルミニウム、亜鉛等との金属との塩などが挙げられる。これらの塩は公知の方法により遊離の本発明ピロロナフチリジニウム誘導体より製造でき、或いは相互に変換することができる。

【0009】また本発明化合物においてシーストランス体、光学異性体、配座異性体等の立体異性体が存在する場合、或いは水和物や錯化合物の状態で存在する場合においても、本発明はそのいずれの立体異性体、水和物、錯化合物をも包含する。

【0010】次に、本発明化合物の製造方法の一例を述べる。 R_1 -NH₂、 R_2 -NH₂、又は R_3 -NH₂で表される化合物(アミン成分： R_1 、 R_2 、及び R_3 は各々前記と同じ基を表す)を、例えばグルコース、フラクトース、ガラクトース、マンノース、デオキシグルコース等の六炭糖、グルコサミン、ガラクトサミン等のアミノ糖、サッカロース等のオリゴ糖などの糖類と共存させることにより、本発明化合物を得ることができる。又、アミン成分として、蛋白質、ペプチド類などを用いて混合反応させた後、酸加水分解処理を行い本発明化合物を得ることもできる。

【0011】反応温度、反応時間、pH等の反応条件に関しては特別な設定条件はなく、適宜設定することができる。操作上簡単なのは室温に放置しておけばよいが、加熱することなどにより反応を促進できる。得られた本発明化合物は、蒸留、クロマトグラフィー、再結晶等の通常的手段により精製することができる。

【0012】

【実施例】以下に本発明ピロロナフチリジニウム誘導体の実施例を示すが、本発明はこれによって限定されるものではない。

実施例1. グルコース79.2gとγ-アミノ酪酸(GABA)45.3gを250mMリン酸緩衝液(pH7.3)1100mLに溶解し、37℃で45日間静置した。反応溶液をスルホン酸型陽イオン交換樹脂/DIAION PK-216(三菱化成)に添加し、水洗後、2Nアンモニア水で溶出した溶液を減圧下40℃水浴中で濃縮乾固した。乾固物を少量のイオン交換水に溶解した後、イオン交換水で平衡化したAMBERLITE XAD-2カラム(オルガノ社)に添加し、通過画分を集めた。この画分を濃縮後、DEVELOSTIL ODS LOP-45Sカラム(野村化学)に添加し、メタノールトリフルオロ酢酸混合溶液で溶出した。さらにこれを逆相高速液体クロマトグラフィー/STR ODS-IIカラム(島津テクノロジー)により分離精製し、立体異性体である化合物1(65mg)、化合物2(182.9mg)及び化合物3(35.5mg)をそれぞれ単離した。

【0013】実施例2. グルコース25.2gとα-ア

セチルリジン 3.4 g を 250 mM リン酸緩衝液 700 mL に溶解し、実施例 1 と同様の操作を行い、立体異性体である化合物 4 (2 mg) 及び化合物 5 (3 mg) をそれぞれ単離した。

【0014】得られた本発明化合物の物性値を以下に示す。尚、上記実施例と同様の方法で 1 位又は全炭素が ^{13}C でラベルされたグルコースを用いてラベル化合物を製造し、構造解析に用いた。蛍光スペクトルは 650-40 Fluorescence Spectrophotometer (日立) により、紫外外部吸収 (UV) スペクトルは DU-650 (Beckman) により共にメタノール中で測定した。スパッタードイオン質量分析ペクトル (SIMS) は M-80B (日立) によりマトリックスにグリセリンを用いて測定した。核磁気共鳴 (NMR) スペクトルは重水中、ARX-500 (Bruker) で測定し、プロトンは共鳴周波数 500.13 MHz を、 ^{13}C は共鳴周波数 125.77 MHz を 0.00 ppm とした。

^1H -NMR スペクトル及び ^{13}C -NMR スペクトルの帰属は ^1H - ^1H COSY、HMQC などの 2 次元 NMR により決定した。

【0015】・化合物 1

8-ヒドロキシ-1, 2, 6, 7, 8, 8a-ヘキサヒドロ-3- (1, 2, 3-トリヒドロキシプロピル)-1, 4, 6-トリス (3-カルボキシプロピル)-ピロロ [2, 3, 4-de] [1, 7] ナフチリジニウム
 蛍光スペクトル: EX_{max} = 370 nm, EM_{max} = 450 nm
 UV スペクトル: λ_{max} = 237, 276, 360 nm
 SIMS: m/z 527

^1H -NMR (D_2O) - δ ppm: 1.79 (2H, m, H-2''), 2.04 (2H, m, H-2'), 2.13 (2H, m, H-2''), 2.33 (2H, t, J=7Hz, H-3''), 2.44 (4H, t, J=7Hz, H-3', 3''), 3.35 (2H, m, H-1''), 3.47 (1H, m, H-1''), 3.50 (1H, dd, J=14, 2Hz, H-7), 3.59 (1H, m, H-1'), 3.63-3.69 (4H, m, H-7, 10, 11, 11), 4.33 (1H, m, H-1''), 4.68 (1H, brs, H-8), 4.68 (1H, m, H-1''), 4.83 (1H, d, J=14Hz, H-2), 4.98 (1H, d, J=1Hz, H-8a), 5.20 (1H, d, J=9Hz, H-9), 5.36 (1H, dd, J=14, 1Hz, H-2), 7.92 (1H, s, H-5)
 ^{13}C -NMR (D_2O) - δ ppm: 22.2 (t, C-2', 2''), 27.1 (t, C-2''), 31.5 (t, C-3''), 32.2 (t, C-3'), 33.3 (t, C-3''), 49.4 (t, C-1''), 53.5 (t, C-7), 54.5 (t, C-1'), 58.6 (d, C-8), 59.8 (t, C-2, 1'), 63.2 (t, C-11), 67.3 (d, C-8a), 68.6 (d, C-9), 74.6 (d, C-10), 126.8 (d, C-5), 134.6 (s, C-2a), 135.3 (s, C-8b), 139.7 (s, C-3), 140.8 (s, C-5a), 178.8 (s, C-4''), 180.2 (s, C-4', 4'')

【0016】・化合物 2

8-ヒドロキシ-1, 2, 6, 7, 8, 8a-ヘキサヒドロ-3- (1, 2, 3-トリヒドロキシプロピル)-1, 4, 6-トリス (3-カルボキシプロピル)-ピロロ [2, 3, 4-de] [1, 7] ナフチリジニウム
 蛍光スペクトル: EX_{max} = 370 nm, EM_{max} = 450 nm
 UV スペクトル: λ_{max} = 240, 277, 360 nm
 SIMS: m/z 527

^1H -NMR (D_2O) - δ ppm: 1.82 (2H, m, H-2''), 2.06 (2H, m, H-2'), 2.12 (2H, m, H-2''), 2.35 (2H, t, J=7Hz, H-3''), 2.46 (4H, t, J=7Hz, H-3', 3''), 3.33 (1H, ddd, J=14, 7, 7Hz, H-1''), 3.42 (1H, ddd, J=14, 7, 7Hz, H-1''), 3.48 (1H, m, H-1'), 3.49 (1H, dd, J=14, 2Hz, H-7), 3.61 (1H, m, H-1'), 3.68 (1H, dd, J=14, 1Hz, H-7), 3.69 (1H, ddd, J=9, 3, 3Hz, H-10), 3.73 (1H, dd, J=3, 3Hz, H-11), 3.76 (1H, dd, J=3, 3Hz, H-11), 4.39 (1H, m, H-1''), 4.68 (1H, d, J=2Hz, H-8), 4.79 (1H, m, H-1''), 4.89 (1H, d, J=14Hz, H-2), 5.01 (1H, d, J=2Hz, H-8a), 5.06 (1H, d, J=14Hz, H-2), 5.21 (1H, d, J=9Hz, H-9), 7.95 (1H, s, H-5)

^{13}C NMR (D_2O) - δ ppm: 21.8 (t, C-2''), 22.2 (t, C-2'), 26.8 (t, C-2''), 31.5 (t, C-3''), 31.8 (t, C-3'), 32.0 (t, C-3''), 49.8 (t, C-1''), 53.9 (t, C-7), 54.3 (t, C-1'), 58.8 (t, C-1''), 60.4 (d, C-8), 61.1 (t, C-2), 63.7 (t, C-11), 67.7 (d, C-8a), 69.0 (d, C-9), 74.8 (d, C-10), 127.3 (d, C-5), 134.5 (s, C-2a), 135.1 (s, C-8b), 141.1 (s, C-3, 5a), 178.0 (s, C-4', 4''), 178.8 (s, C-4'')

【0017】・化合物 3

8-ヒドロキシ-1, 2, 6, 7, 8, 8a-ヘキサヒドロ-3- (1, 2, 3-トリヒドロキシプロピル)-1, 4, 6-トリス (3-カルボキシプロピル)-ピロロ [2, 3, 4-de] [1, 7] ナフチリジニウム
 蛍光スペクトル: EX_{max} = 373 nm, EM_{max} = 452 nm
 UV スペクトル: λ_{max} = 239, 278, 360 nm
 SIMS: m/z 527

^1H -NMR (D_2O) - δ ppm: 1.84 (2H, m, H-2''), 2.03 (2H, m, H-2'), 2.12 (2H, m, H-2''), 2.35 (2H, t, J=7Hz, H-3''), 2.46 (4H, m, H-3', 3''), 3.31 (1H, ddd, J=14, 7, 7Hz, H-1''), 3.39 (1H, ddd, J=14, 7, 7Hz, H-1''), 3.46 (1H, dd, J=14, 1Hz, H-7), 3.50 (1H, m, H-1'), 3.78 (1H, dd, J=14, 3Hz, H-7), 3.70-3.79 (4H, m, H-10, 11, 11, 1'), 4.32 (2H, m, H-8, 1''), 4.78 (1H, m, H-1''), 4.79 (1H, d, J=10Hz, H-8a), 4.83 (1H, d, J=14Hz, H-2), 5.08 (1H, d, J=14Hz, H-2), 5.22 (1H, d, J=9Hz, H-9), 7.93 (1H, s, H-5)

^{13}C -NMR (D_2O) - δ ppm: 21.8 (t, C-2''), 22.1 (t, C-2'), 26.6 (t, C-2''), 31.3 (t, C-3''), 31.6 (t, C-3'), 31.8 (t, C-3''), 49.6 (t, C-1''), 54.0 (t, C-7), 55.7 (t, C-1'), 60.2 (t, C-2), 60.4 (t, C-1'), 63.5 (t, C-11), 63.9 (d, C-8), 69.0 (d, C-9), 70.4 (d, C-8a), 74.7 (d, C-10), 127.7 (d, C-5), 134.1 (s, C-2a), 135.2 (s, C-8b), 141.4 (s, C-5a, 3), 177.7 (s, C-4''), 177.7 (s, C-4'), 178.8 (s, C-4'')

【0018】・化合物 4

8-ヒドロキシ-1, 2, 6, 7, 8, 8a-ヘキサヒドロ-3- (1, 2, 3-トリヒドロキシプロピル)-1, 4, 6-トリス [6- (N-アセチル-L-ノルロイシル)]-ピロロ [2, 3, 4-de] [1, 7] ナフチリジニウム
 蛍光スペクトル: EX_{max} = 374 nm, EM_{max} = 452 nm
 UV スペクトル: λ_{max} = 237, 277, 360 nm

S I M S : m/z 782

¹H-NMR(D₂O) - δ ppm: 1.40(6H, m, H-3', 3'', 3'''), 1.68(2H, m, H-2''), 1.72(4H, m, H-2', 2''), 1.84(6H, m, H-4', 4'', 4'''), 1.98(3H, s, AcO''-Me), 1.99(3H, s, AcO'-Me), 2.00(3H, s, AcO''-Me), 3.41(1H, m, H-1'), 3.53(2H, m, H-1''), 3.61(1H, dd, J=14, 1Hz, H-7), 3.62(1H, m, H-1'), 3.74(1H, dd, J=14, 1Hz, H-7), 3.79(3H, m, H-10, 11, 11'), 4.23(3H, m, H-5', 5'', 5'''), 4.42(1H, m, H-1''), 4.70(1H, s, H-8), 4.70(1H, m, H-1'), 4.89(1H, d, J=14Hz, H-2), 5.07(1H, d, J=1Hz, H-8a), 5.22(1H, d, J=9Hz, H-9), 5.41(1H, dd, J=14, 1Hz, H-2), 7.95(1H, s, H-5)

¹³C-NMR(D₂O) - δ ppm: 22.6(q, AcO'-Me, AcO''-Me, AcO'''-Me), 22.8(t, C-2'), 23.1(t, C-2'', 3', 3'''), 25.4(t, C-3''), 26.0(t, C-2''), 30.8(t, C-4'), 30.9(t, C-4''), 31.3(t, C-4'') 49.9(t, C-1'), 53.4(d, C-5', 5''), 53.6(d, C-5''), 54.6(t, C-7, 1''), 58.5(d, C-8), 60.5(t, C-1''), 60.8(t, C-2), 63.1(t, C-11), 67.3(d, C-8a), 68.6(d, C-9), 74.4(d, C-10), 126.6(d, C-5), 134.0(s, C-2a), 134.3(s, C-8b), 140.1(s, C-3), 140.7(s, C-5a), 175.1(s, C-6'), 175.1(s, C-6''), 175.2(s, C-6''), 176.6(s, AcO'-CO), 176.7(s, AcO''-CO), 176.8(s, AcO'''-CO)

【0019】・化合物5

8-ヒドロキシ-1, 2, 6, 7, 8, 8a-ヘキサヒドロ-3-(1, 2, 3-トリヒドロキシプロピル)-1, 4, 6-トリス[6-(N-アセチル-L-ノルロイシル)]-ピロロ[2, 3, 4-de][1, 7]ナフチリジニウム

蛍光スペクトル: EX_{max} = 376 nm, EM_{max} = 452 nmUVスペクトル: λ_{max} = 239, 278, 360 nm

S I M S : m/z 782

¹H-NMR(D₂O) - δ ppm: 1.39-1.48(6H, m, H-3', 3'', 3'''), 1.66(2H, m, H-2''), 1.73(4H, m, H-2', 2''), 1.89(6H, m, H-4', 4'', 4'''), 1.98(3H, s, AcO''-Me), 1.98(3H, s, AcO'-Me), 2.00(3H, s, AcO''-Me), 3.39(1H, m, H-1''), 3.44(1H,

m, H-1''), 3.50(1H, m, H-1'), 3.57(1H, dd, J=14, 2Hz, H-7), 3.65(1H, m, H-1'), 3.73(1H, dd, J=14, 1Hz, H-7), 3.81(3H, m, H-10, 11, 11'), 4.27-4.35(4H, m, H-1'', 5', 5'', 5'''), 4.75(1H, hrs, H-8), 4.78(1H, m, H-1''), 4.94(1H, d, J=14Hz, H-2), 5.08(1H, hrs, H-8a), 5.09(1H, dd, J=14, 1Hz, H-2), 5.20(1H, d, J=9Hz, H-9), 7.95(1H, s, H-5)

【0020】

【発明の効果】AGEとしては幾つかの候補物質がこれまでに報告されており現在も研究が進められている。本発明者やその共同研究者らも、特開平6-73057号及び特開平8-48686号で開示されているようなピリジニウム誘導体やナフチリジニウム誘導体を見出し、これらはジアミンの縮合二環系を基本母核としていた。本発明ピロロナフチリジニウム誘導体は従来の化合物とは異なり、トリアミンの縮合三環系を基本母核とするものであり、生体内蛋白質との架橋可能な部位が3ヶ所あるというその新規な構造から、全く新しいAGE候補物質として非常に注目すべきものである。

【0021】従って、従来報告されてきたAGE化合物と同様に、本発明化合物を指標として、糖尿病及び糖尿病性腎症、糖尿病性動脈硬化症、糖尿病性神経障害、糖尿病性白内障、糖尿病性網膜症、糖尿病性細小血管障害等の糖尿病性合併症並びに老化やそれに伴う疾患等の診断が可能であり、さらにインビトロ及びインビボ試験系において本発明化合物を指標として薬効評価等を行うことができる。また、本発明化合物をハプテンとして作成された抗体は、前述の診断や薬効評価において免疫化学的且つ免疫組織化学的に利用でき、非常に有用性が高い。上述したように本発明ピロロナフチリジニウム誘導体は既知のAGEとは明らかに異なるトリアミンの縮合三環系骨格である新規物質であり、これまでのAGE候補物質とは違った生体内での存在や生物活性が示唆され、異なった有用性も期待できる。